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CONTROL OF LOOSE SMUT OF BARLEY

by

Richard Mullington Lewis

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

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Approved:

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Ames, Iowa

1962

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INTRODUCTION

Control of Ustilago nuda (Jens.) Rostr., the causal organism of loose smut of barley is difficult. Infection occurs at the time of flowering, the mycelium becoming established in the pericarp and certain portions of the embryo. Infected seeds cannot be differentiated macroscopically from non-infected seeds. At the time of germination the mycelium becomes systemic in the plant, eventually replacing completely the floral primordia and giving rise to teliospores in these portions of the host.

Surface disinfestation by fungicides does not control the disease because the pathogen is within the seeds. A treatment to be successful must penetrate host cells and be differentially selective against the pathogen mycelium with minimal injury to host cells. The modified classic hot water treatment consisting of a 6-hour presoak, in some treatments followed by a prewarming dip in warm water (45°C), then immersion for 15 minutes in hot water (52°C), requires careful temperature control. The disease can be controlled by this treatment, but the grain is left wet and swollen and a sizeable stand reduction often occurs. Wet grain must be planted immediately with specially calibrated drills or carefully dried for storage. The problem of wet grain exists for all seed treatments in which much water is added.

The soak method, more recently developed, does not require the critical temperature control of the hot water method; but like the hot water method, does result in some reduction in seedling emergence. The reduction in some cases is not great but the wet seed is still objectionable. The anaerobic method also has the disadvantage of wet seed. Another disadvantage in some of these treatments (the long soak in water alone and the anaerobic) is the failure to control some other seed borne pathogens. Also, with no fungicide residue on the seed surface no protection is provided against seed rotting organisms in the soil.

The object of this research has been to investigate the possibilities of low moisture fungicide treatments for controlling loose smut in barley. The low moisture level requires time in storage to permit the fungicide and solvent to penetrate the seed and the moisture added is not sufficient to require special handling. Furthermore, the grain is left with a protective fungicide coating.

PERTINENT LITERATURE

The first effective control of loose smut of barley was the hot water (55.6°C) treatment developed by J. L. Jensen (6) in 1887. To prevent cooling of the final treatment water, several dips in two separate vessels of water at 55.6°C preceded the final treatment consisting of three 1-minute dips in water at the initial temperature. Seed was then dried.

Jensen (7) in 1890 modified the hot water treatment to include a 4-hour cold water soak followed by a 4-hour period during which the seed remained in the wet sack and drained. The seed was then given prewarming dips in water at 43.3 - 48.9°C . The final treatment was a 5-minute immersion in water at 52.2 - 53.3°C . Since that time the method has undergone many other modifications of water temperatures and immersion times. Chemicals have been added to the water and the procedures for drying treated seed have been improved. These modifications were designed to achieve control without depending upon critical water temperatures and soaking times, and to reduce seed injury due to treatment. Further investigations on control of loose smut of barley using surface as well as short-soak applications of fungicides, were developed through 1956 (3).

Aqueous soaks at room temperature of inorganic, organic and metallo-organic compounds in addition to two antibiotics

for control of loose smut were investigated in 1951 by Tyner (12). Seed was given a presoak followed by soaks of 18 to 40 hours in the treatment materials. Satisfactory control was obtained with solutions of 0.5 per cent boric acid, 0.1, 0.2, and 0.3 per cent Spergon and with 0.2 per cent potassium iodide. A 6-hour presoak followed by a 40-hour soak in a 0.2 per cent aqueous suspension of Spergon resulted in complete control of loose smut and was considered the best treatment.

Again in 1952 the best control of smut was obtained with Spergon treatments (15). Germination averaged 63 per cent, ranging from 35 to 93 per cent with only two of the six barley varieties tested germinating under 50 per cent. Though control of loose smut by water soaks alone was obtained by Tyner (13, 14), his results indicated that Spergon reduced the treatment time required for elimination of smut. The concentrations of Spergon used also resulted in some suppression of fermentation which was a problem in soaks using water alone.

In the anaerobic method for control of loose smut of barley, developed by T. T. Hebert (4) in 1955, seed was first given a 6-hour presoak, drained, and then hermetically sealed. Control of smut was obtained at 32°C in 22 hours, at 28°C in 31 hours, and at 24°C in 42 hours. Hebert (5) postulated the control was due to the inability of the fungus to survive

after respiration of the soaked seeds had removed the oxygen from the atmosphere of the sealed container. The anaerobic treatment followed with a fungicide to control disease-causing organisms on the seed surface is recommended for general use (11). A seed treatment device developed in 1960 simplified the anaerobic method by utilizing an oil drum so modified that seed could be soaked, drained, given sealed storage and dried without being removed from the container (9).

Reduction of loose smut was obtained in 1956 with Panogen and Spergon applied in 2-hour soaks followed by sealed storage for 23 hours (10). Elimination of the disease probably would have resulted with longer storage. Panogen 0.15 ml. with 10 ml. of water and Panogen at the same rate with 20 ml. of water after 88 hours of sealed storage reduced the disease to 1 smutted head per row while the controls had a mean of 6 smutted heads. The advantages of Panogen are ease of dilution in water and control of covered smut and stripe. It was recommended also as a partial control of seedling blight and root rot caused by Helminthosporium sativum Pam., King, and Bakke. Panogen contains an organic mercury and the value of organic mercury fungicides in treatment of cereal diseases has been well established (3).

Even though the slurry method is used in treating cereals for control of surface borne fungi (2), it has not been investigated as a control for loose smut of barley.

MATERIALS AND METHODS

Materials

Two kinds of materials were used in this investigation; barley seed infected with the loose smut fungus and chemicals with fungicidal properties. A sufficient quantity of barley seed with an adequate level of loose smut infection is often difficult to obtain. The seed used in these experiments, with the exception of 1955, was obtained from the Agronomy Department of Iowa State University, Ames, Iowa, through the courtesy of Dr. R. E. Atkins and Dr. K. J. Frey (Table 1) as remnants from lots of barley which were expected to have a fair level of smut. The Mars barley used in 1952 and 1953 was a remnant from seed which averaged 38 smutted heads per rod row in the field in 1951. No loose smut counts from prior years were available for seed used in 1954 and 1955.

Since organic mercuries have been outstanding in control of surface borne cereal smuts, they were used in this investigation. Most of the compounds used were supplied to Dr. C. S. Reddy¹ by the manufacturer and many were coded experimentals. The mercury injury inhibitors were unlabeled experimental materials supplied by Dr. Reddy and were indicated as inhibitors A and B. Recommendations of the manufacturer were used where available as a starting point for dosages.

¹Dr. C. S. Reddy, Professor, Department of Botany and Plant Pathology, Iowa State University, Ames, Iowa.

Table 1. Varieties and sources of barley seed used in loose smut experiments

Year used	Variety of barley	Source
1952	Mars	Agronomy Department, Iowa State University, Ames, Iowa. Seed remnants from 1950.
1953	Mars	"
1954	Montcalm Moore Wisconsin 38 (a mixture of one part of each variety was used)	Agronomy Department, Iowa State University, Ames, Iowa. Seed collected from a 1953 loose smut experiment.
1955	Kindred L.	Marlow Yunker Farm, Rock Valley, Iowa. 1953 remnant.
	Mixed barley varieties (Montcalm, Moore, Wisconsin 38)	Increased at Northern Iowa Agricultural Experimental Association Farms, Kanawha, Iowa, 1954. Spray inoculated with an aqueous suspension of loose smut spores at time of flowering.

Methods of Application

Two methods for applying the chemicals were used; the aqueous soak and the slurry. The soak method was investigated because of current interest and was used in preliminary tests to determine the effect of potential treatment materials on seed viability. The slurry method, which utilizes a small

Table 2. Chemicals used to control loose smut of barley

Trade name or code number	Chemical	Manufacturer
Acetone	Dimethyl ketone	-----
BB-71 (liquid)	Experimental mercury seed treatment	Unknown origin
C-6146	Experimental compound	Union Carbide and Carbon Corp.
Ceresan M	7.7 per cent Ethyl mercury-p-toluene sulfonanilide	I. E. du Pont de Nemours & Co., Inc.
Ceresan 2 per cent	2 per cent Ethyl mercury chloride	"
du Pont Liquid 244	2.27 per cent Phenyl mercury acetate 1.57 per cent Ethyl mercury acetate	"
E.C. 1182	3.8 per cent 4-chloro- 3,5-dimethylphenoxy ethanol	Union Carbide and Carbon Corp.
8-Hydroxy- quinolate sulfate	Same	-----
Inhibitor A	Experimental mercury injury inhibitor ^a	-----
Inhibitor B	"	-----
N-244	3-p-chlorophenyl- 5-methyl rhodanine	Stauffer Chemical Co.
N-521	3,5-dimethyl-tetra- hydro-1,3,5, 2H- thiadiazine-2-thione	"

^aSupplied by Dr. C. S. Reddy.

Table 2 (Continued).

Trade name or code number	Chemical	Manufacturer
Panogen S Liquid	Unknown	Morton Chemical Co. (formerly Panogen, Inc.)
Panogen Liquid Seed Disin- fectant	2.2 per cent Methyl- mercury-dicyan-diamide	"
P.D.R. "C" ^b	175 g. Ceresan 2 per cent + 200 g. Inhibi- tor A	-----
P.D.R. "Regular" ^c	175 g. Ceresan 2 per cent + 140 g. Inhibi- tor A	-----
Phillips OSS- 11148	Mercury tertiary butyl mercaptide	Phillips Petroleum Co.
Phygon XL	50 per cent 2,3-Dichloro-1,4- naphthoquinone	United States Rubber Co.
Puratized C4-10	Experimental mercury seed treatment	Gallowhur Chemical Corp.
Puratized C13-1212	Experimental seed treatment	"
Puratized C15-127	"	"
P.M.A.S.	7 per cent Phenyl- mercury acetate	W. A. McCleary Corp.
Spargon 96 per cent	96 per cent Tetra- chloro-p-benzoquinone	United States Rubber Co.

^bA seed potato treatment modified by Dr. C. S. Reddy.

^cSeed potato treatment formulated by Dr. C. S. Reddy.

Table 2 (Continued).

Trade name or code number	Chemical	Manufacturer
Spergon 46 per cent wettable	46 per cent Tetra- chloro-p-benzoquinone	United States Rubber Co.
Triton-B-1956 (emulsifier B)	Phthalic glycerol alkyl resins	Rohm and Haas Chemical Co.
Velsicol 50-C5-46	N-Ethylmercury-1,2,3, 6-tetrahydro-3:6 endomethane-3,4,5,6, 7,7-hexachloro- phthalimide	Velsicol Corp.

amount of solvent to aid in absorption of the chemical, was of the greatest interest in this investigation. With this method it is possible to avoid the disadvantages of wet, swollen seed and the need for an additional seed treatment to control surface borne pathogens.

Soak method

In the soak method the chemical was added to water and thoroughly mixed, emulsifiers being added in some treatments to facilitate suspension of the chemical. Weighed grain samples in cheesecloth sacks were suspended in the solution for soak periods of 10, 24, and 46 hours at room temperature. Soaked seeds were dried as rapidly as possible under an electric fan at room temperature. Soak treatment dosages

are given as parts of the chemical to parts of water; for example 8:1,000 indicates 8 parts of the chemical are used to 1,000 parts of water.

Slurry method

In the slurry method chemicals were applied with a solvent which was added to the seed sample at the rates of 2.5 to 15.6 per cent of the weight of the seed sample. A solvent addition of 5 per cent indicates that 5 ml. of the solvent has been added to a 100 g. sample of barley seed (5 per cent of the seed weight). "Total Moisture Added" is abbreviated in the text and tables as T.M.A. The phrase indicates that all or a part of the solution added was a saturated solution of one of the inhibitors. Water, acetone, or acetone and water solutions were used as solvents. Dosages are given in grams or milliliters per 100 g. of seed. Where recommendations were available from the manufacturer the dosages in ounces per bushel as well as in grams per 100 g. of seed are given in the Appendix under the chemical.

Chemicals were applied in Mason jars used also for storage. By capping the jars and shaking manually, the chemicals were distributed on the inner surfaces. The solvent aliquots were added, the jars again shaken, and then the seed added, followed by another shaking. The jars were then placed on moving rollers for 30 minutes or until all evidence of moisture on the walls had disappeared. The lids

were tightened and the jars stored at room temperature until seed was sampled for greenhouse emergence tests or packaged for field planting. Treatment modifications are given in the Appendix with the specific treatments.

Most slurry treated seeds were in sealed storage for at least 90 days prior to field planting. The exact storage times are given in the Appendix with treatment dosages and the amounts of reduction of loose smut in the field.

Seed Treatment Evaluations

Greenhouse seedling emergence tests

Greenhouse seedling emergence tests were used to determine the effects of the treatment on seed viability. If emergence was below an average of 30 seedlings after 14 to 15 days of sealed storage the treatments were either discarded or modified. Emergence tests were made on all slurry treated seed except the 1952 material but not on soak treatments prepared for the field.

Treatment sample sizes used in greenhouse emergence tests were 3 g. in 1953, 2.7 g.^a in 1954, and 100 seeds in 1955. Treatments were replicated twice except in 1953 where only one sample per treatment was planted. Samples were planted in greenhouse benches filled with coarse sand which were routinely used for other seed treatment emergence tests.

^aAn average weight for four 100 seed samples.

Seedling counts were made 9 days following planting except where otherwise indicated.

Field procedures

Seed samples for the field experiments were weighed and placed in marked coin envelopes. Treatments containing mercury compounds were separated from other treatments until arranged in the field for planting. All samples were a minimum of 15 g. which would permit approximately 1 g. of seed per foot in a 15 foot row (rod row). If more seed was available longer rows were planted, then trimmed to 15 feet after seedlings had emerged. Seeds were planted manually using a funnel drop planter.

Experiments were planted in the field as soon as conditions permitted except in 1952 when some treatments were planted late. All experiments were in the small grain nursery of the Northern Iowa Agricultural Experimental Association Farm at Kanawha, Iowa, except for two replicates in 1952 which were grown at Ames, Iowa.

The field experiments in 1952, 1953, and 1954 were not laid out in an experimental plot design, treatments being non-randomized with controls usually every tenth row. The field experiment in 1955 was laid out in a split plot design. Table 3 gives the planting dates, sample sizes and numbers of replicates used in all field experiments.

Table 3. Replications, sample sizes, dates of planting and smut counts for all field experiments

Year	Treatment sample size in grams	Number of replications	Field planting date	Date of smut count
1952				
Soak	20	4	4/19	6/24
Slurry	20	2	5/24	7/2
1953	15	3	5/5	7/22
1954	15	5	4/12	6/22
1955	18	12	4/16	6/20

Collection and evaluation of loose smut data

Counts of smutted heads per rod row were made as soon as possible after appearance of the smut. Loose smut is recorded in the tables as the percentage reduction of loose smut calculated as follows. The average number of smutted heads per row for a treatment was divided by the average number of smutted heads per row for the dry untreated control. The resulting loose smut percentage was then subtracted from one hundred giving the reduction of loose smut in comparison to that in the dry untreated control.

EXPERIMENTAL RESULTS

Field tests of treatments designed to control loose smut were made four years: 1952, 1953, 1954, and 1955. Only the first two years included soak treatments, the principal interest being to develop a slurry treatment control. Results are presented for the four years of experimentation.

1952 Results

Soak treatments in 1952 were selected from preliminary dosage level experiments using various soak periods and concentrations. These tests were used to screen materials for effect on seed viability as measured by greenhouse seedling emergence. Some slurry treatments were also used.

The dry untreated controls for the soak treatments, replicated six times, averaged 22.5 smutted heads per rod row with a range of 3 to 35 smutted heads. The dry untreated control for the slurry treatments, replicated four times, averaged 5.5 smutted heads per rod row with a range of 3 to 11 smutted heads. The same lot of Mars barley was used for all treatments and controls; however, planting dates were different (Table 3). Environmental differences, such as soil temperature, more favorable for rapid growth of the barley plant than for the loose smut mycelium, may have permitted some floral primordia of infected plants to escape infection.

Reduction of smut incidence in plants from the same seed lot, such as occurred, could have resulted from environmental variation.

Treatments which provided at least 91 per cent reduction of loose smut were considered to be potentially effective control materials for field use. They were used in subsequent experiments. Other treatments which did not give this level of control, but were of interest experimentally for various reasons, were also utilized in further tests.

Soak treatments

Satisfactory reduction of loose smut was not obtained from any of the 10 and 24-hour soak treatments. However, the greatest reduction of loose smut, 88.7 per cent, was obtained using Spergon 96 per cent at 4:1,000 with the addition of an emulsifier in a 24-hour soak (Treatment 52-8). Reduction of 87.6 per cent using P.D.R. "C" (Treatment 52-3) and 84 per cent using P.D.R. "Regular" (Treatment 52-4) occurred with a 24-hour soak period. From 40 to 77 per cent reduction of loose smut resulted from Treatments 52-1, 52-2, 52-5, 52-6, and 52-7, Table 4.

Slurry treatments

Complete control of loose smut was obtained by one slurry treatment (52-11) using P.D.R. "Regular" at 0.06 g. with a 1:1 acetone and water solvent added. Other treatments

Table 4. Reduction of loose smut in barley grown from soak-treated seed, 1952

Treatment number	Treatment	Soak period in hours	Percentage reduction of loose smut
	Control - dry untreated seeds	--	0 ^a
52-1	C-6146 9:10,000 ^b	24	66
52-2	Inhibitor A 50 per cent 1:1,000	24	77
52-3	P.D.R. "C" 1:1,000	24	87.6
52-4	P.D.R. "Regular" 1:1,000	24	84
52-5	P.D.R. "Regular" 1:800	24	73
52-6	P.D.R. "Regular" 1:400	10	73
52-7	Phygon XL 1:375	24	40
52-8	Sperguson 96 per cent 4:1,000 ^c	24	88.7

^aThe loose smut average of 22.2 heads per rod row in the control is based on a six replicate average of dry untreated Mars barley. Loose smut ranged from 3 to 35 heads per row.

^bEmulsifier B (Triton-B-1956) added at the rate of 9:10,000.

^cEmulsifier B added at the rate of 0.08:1,000.

Table 5. Reduction of loose smut in barley grown from slurry-treated seed, 1952

Treatment number	Treatment	Percentage reduction of loose smut
	Control - dry untreated seeds	0 ^a
52-9	Ceresan M 0.0651 g.	8
52-10	E.C. 1182 and acetone sol. 6:10,000, 10 ml.	0
52-11	P.D.R. "Regular" 0.06 g. + 5 per cent 1:1 acetone and water sol.	100
52-12	P.M.A.S. 0.13 ml. + 3 per cent water	0

^aThe average number of smutted heads in four replicates of the dry Mars barley control was 5.5 heads per rod row. The number of smutted heads ranged from 3 to 11 heads per row.

did not provide satisfactory reductions in loose smut,
Table 5.

1953 Results

Slurry treatments, as a means of controlling loose smut, were studied more intensively in 1953 because of the inherent advantages such treatments have over currently used methods of control. A few soak treatments were carried for comparisons. Treatments which resulted in less than 91 per cent reduction of loose smut have been omitted from Tables 6 and 7.

They are presented in the Appendix. The dry untreated controls for both the soak and the slurry treatments averaged 22.6 smutted heads per rod row in 18 replicates with a range of 7 to 40 smutted heads per row.

Soak treatments

All soak treatments in 1953 resulted in 87 per cent or greater reduction of loose smut (Table 6). Elimination of loose smut was obtained using Spergon 46 per cent wettable at 2:1,000 in a 6-hour presoak, followed by a 40-hour soak in the chemical (Treatment 53-2). A 91 per cent reduction of loose smut resulted from a single 46-hour soak in water alone; therefore, part of the reduction from Treatment 53-2 can be attributed to the long water soak rather than to the effects of Spergon. A 97 per cent reduction of loose smut resulted from P.D.R. "C" 1:1,000 used in a 24-hour soak (Treatment 53-1 b). The same treatment in the previous year (Treatment 52-3) reduced loose smut only 87.6 per cent. Spergon 96 per cent, 4:1,000 in a 24-hour soak (Treatment 53-3) reduced loose smut 87 per cent in 1953, about the same as in 1952 (Treatment 52-8).

Slurry treatments

Loose smut was eliminated by the following four treatments and reduced to 99 per cent in a fifth (Table 7):

1. Ceresan M at 0.13 g. with 5 per cent addition of 1:1 acetone and water (Treatment 53-21);

Table 6. Reduction of loose smut in barley grown from soak-treated seed, 1953

Treatment number	Treatment	Soak period in hours	Percentage reduction of loose smut
	Control - dry untreated seeds	--	0 ^a
53-1	Water	24	72
53-1 a	Water	46	91
53-1 b	P.D.R. "C" 1:1,000	24	97
53-3	Spergon 96 per cent 4:1,000 ^b	24	87
53-2	Spergon 46 per cent wettable 2:1,000	6-hr. presoak 40-hr. in chem.	100

^aThe percentage reduction of loose smut was based on the average of 22.6 smutted heads per rod row in the dry untreated control.

^bEmulsifier B (Triton-B-1956) was added at the rate of 2 per cent of the Spergon weight.

2. Ceresan M at 0.13 g. with a saturated solution of Inhibitor A and 15.6 per cent T.M.A. (Treatment 53-24);
3. Ceresan M at 0.26 g. with 5 per cent water added (Treatment 53-27);
4. Ceresan M at 0.26 g. with Inhibitor A and 5 per cent water added (Treatment 53-28);
5. Velsicol 50-C5-46 at 0.26 g. with 5 per cent water added, (Treatment 53-61), 99 per cent reduction.

Loose smut was reduced 96 per cent with Ceresan M 0.13 g. plus a saturated solution of Inhibitor B (Treatment 53-25) and with Phillips OSS-11148 at two dosage levels (Treatments 53-44 and 53-45). A reduction of 93 per cent was obtained with three treatments; Acetone 15 ml. (Treatment 53-5), Ceresan M 0.065 g. plus a saturated solution of Inhibitor B and acetone (Treatment 53-18) and Ceresan M 0.13 g. plus Inhibitor A and 5 per cent water (Treatment 53-22). A 91 per cent reduction in smut resulted from the following treatments:

1. Ceresan M 0.065 g. with 5 per cent of 1:1 acetone and water solution added (Treatment 53-13);
2. Ceresan M 0.13 g. plus 5 per cent water (Treatment 53-20);
3. Ceresan M 0.13 g. with the addition of Inhibitor A plus 5 per cent of a 1:1 acetone and water solution (Treatment 53-23);
4. Ceresan M 0.26 g. plus a dilution of a saturated solution of Inhibitor B. 5 per cent T.M.A. (Treatment 53-29);
5. Puratized C4-10, 0.195 g. plus 5 per cent of a 1:1 acetone and water solution (Treatment 53-52).

Greenhouse emergence for Treatment 53-24 with 15.6 per cent T.M.A. appeared good in one replication. Further investigations indicated, however, that 15 per cent moisture caused a more rapid loss of viability, measured by greenhouse emergence, than did lower levels of added moisture. Greenhouse seedling emergence data (Table 7), although from only one replicate, indicate a reduction of injury in the organic

Table 7. Greenhouse emergence and percentage reduction of loose smut in barley grown from slurry-treated seed, 1953

Treatment number	Treatment ^a	Days stored prior to planting	Greenhouse emergence ^b	Percentage reduction of loose smut
	Control - dry untreated seeds	--	88	0 ^c
53-4 a	5 per cent water ^d	81	68	32
53-5	Acetone 15 ml.	81	24	93
53-44	Phillips OSS-11148 0.13 g. + 5 per cent water	90	33	96
53-45	Phillips OSS-11148 0.521 g. + 5 per cent water	90	52	96
53-52	Puratized C4-10 0.195 g. + 5 per cent 1:1 acetone and water	90	31	91
53-61	Velsicol 50-C5-46 0.26 g. + 5 per cent water	92	50	99

^aDosages are given in milliliters or grams per 100 g. of seed. For further details refer to the Appendix.

^bGreenhouse emergence is the actual seedling count on only a single replicate.

^cThe average number of smutted heads per rod row for 18 replications was 22.6 heads per rod row; a range of 7 to 40 heads.

^dSolvents added equal to a percentage of the sample weight.

Table 7 (Continued).

Treatment number	Treatment ^a	Days stored prior to planting	Green-house emergence ^b	Percentage reduction of loose smut
	Ceresan M 0.065 g. +			
53-13	5 per cent 1:1 acetone and water	95	51	91
53-18	Inhib. B sat. sol. 2.5 ml. (0.0509 g.) ^e + acetone 2.5 ml.	95	51	93
	Ceresan M 0.13 g. +			
53-20	5 per cent water ^f	93	63	91
53-21	5 per cent 1:1 acetone and water	95	30	100
53-22	Inhib. A dry 0.1042 g. + 5 per cent water	93	73	93
53-23	Inhib. A dry 0.1042 g. + 5 per cent 1:1 acetone and water	95	58	91
53-24	Inhib. A sat. sol. (0.8083 g.) ^e 15.6 per cent T.M.A. ^g	92	70	100
53-25	Inhib. B sat. sol. (0.102 g.) ^e 5 per cent T.M.A.	95	36	96

^eThe approximate amount of actual inhibitor added.

^fAdded with treatment 24 hours prior to addition of seed.

^gT.M.A., total moisture added, whether from the saturated solution alone or with the addition of distilled water.

Table 7 (Continued).

Treatment number	Treatment ^a	Days stored prior to planting	Green-house emergence ^b	Percentage reduction of loose smut
	Ceresan M 0.26 g. +			
53-27	5 per cent water	93	38	100
53-28	Inhib. A 0.2038 g. + 5 per cent water	93	57	100
53-29	Inhib. B dilution from sat. sol. ^h 5 per cent T.M.A.	92	57	91

^hSee the Appendix.

mercury treatments where an injury inhibitor was used (Treatment 53-22 vs. Treatment 53-20; Treatment 53-21 vs. Treatment 53-23; Treatment 53-27 vs. Treatment 53-28).

1954 Results

All 1954 treatments were slurries of organic mercury compounds with various combinations of mercury injury inhibitors and moisture levels. Besides controlling loose smut, greenhouse seedling emergence was of special interest. Since storage time is necessary to allow the treatment to diffuse into the seed, effects of the treatment and time in sealed storage on seed viability become important. A treatment which reduces stand below certain limits is obviously of no practical value.

Reduction of loose smut

Reduction of loose smut ranged from 100 per cent to approximately the level of the dry untreated controls which

averaged 11.3 smutted heads per row (an average of 15 replicates in which loose smut ranged from 5 to 19 heads per row). Treatments which reduced loose smut 91 per cent or better are given in Table 8.

Elimination of loose smut resulted with Ceresan M 0.13 g. plus a saturated solution of Inhibitor A and 5 per cent total moisture added (Treatment 54-33). The same dosage of Ceresan M plus a saturated solution of Inhibitor B with 2.5 per cent total moisture added reduced loose smut 91.2 per cent (Treatment 54-35). Treatment 54-43 using Inhibitor A alone at the same rate as in Treatment 54-33 reduced loose smut 32.8 per cent while treatment 54-54 with Inhibitor B at the same rate as in Treatment 54-35 reduced loose smut 18.6 per cent¹. These data indicate some reduction in loose smut can be attributed to the inhibitors. A smut reduction of 98.2 per cent was obtained with Ceresan M 0.13 g. plus 5 per cent water (Treatment 54-29). The same Treatment (53-20) in 1953 reduced loose smut 91 per cent. The difference in seedling emergence between Treatment 54-33 with the inhibitor and Treatment 54-29 without, was probably not significant (Table 8).

Effects of inhibitors, moisture and storage on greenhouse emergence

An optimum ratio of the mercury injury inhibitor to the mercury containing compound which should result in the

¹See the Appendix for treatment details.

Table 8. Greenhouse seedling emergence and percentage reduction of loose smut in barley grown from slurry-treated seed, 1954

Treatment number	Treatment ^a	Days stored prior to planting	Greenhouse emergence ^b	Percentage reduction of loose smut
	Control - dry untreated seeds	---	83	0
54-1	5 per cent water ^c	103	86	27.4
54-29	Ceresan M 0.13 g. + 5 per cent water	103	70	98.2
54-33	Ceresan M 0.13 g. Inhib. A sat. sol. (0.0665 g.) ^d + 5 per cent T.M.A. ^e	103	75	100
54-35	Ceresan M 0.13 g. Inhib. B sat. sol. (0.1128 g.) ^d + 2.5 per cent T.M.A.	103	87	91.2

^aDosages per 100 g. of seed.

^bEmergence is average of plants in two replicates.

^cAmount of water added as a percentage of seed weight.

^dThe approximate amount of actual inhibitor added.

^eT.M.A. is total moisture added which may be in part from the saturated solution of the inhibitor. The same abbreviation is used in all tables.

greatest injury inhibition apparently exists. The effect of mercury injury inhibition was greatest with the Inhibitor A to Ceresan M ratio of 1.6:1 and with the highest Ceresan M

Table 9. Greenhouse seedling emergence from barley seed given varying periods of storage following slurry treatments of inhibitor A and Ceresan M, 1.6:1 with 5 per cent water added, 1954

Treatment number	Treatment ^a	Days stored prior to planting			
		14	60	102	122
	Control - dry untreated seeds	84	84	86	82
54-1	5 per cent water	88	86	74	82 ^c
54-42	Inhib. A 0.21 g. + 5 per cent water	82	86	77	68 ^c
	Inhib. A to Ceresan M, 1.6:1 + 5 per cent water				
54-14	Ceresan M 0.032 g.	87	88	85	86 ^c
54-23	" 0.065 g.	82	85	84	79 ^c
54-32	" 0.13 g.	73	86	72	63 ^c

^aDosages per 100 g. of seed.

^bEmergence, an average of plants in two replicates except "c".

^cEmergence in only one replicate due to erratic germination.

dosage (Table 9, Treatment 54-32 vs. Treatment 54-29, Table 10). When compared to the same dosage levels of Ceresan M without the inhibitor (Table 10), the effects of injury inhibition (Table 9) become more evident as time in storage and the Ceresan M dosage increases.

Table 10. Greenhouse seedling emergence from barley seed given varying periods of storage following slurry treatments of Inhibitor A and Ceresan M, 1:1, with 5 per cent water added, 1954

Treatment number	Treatment ^a	Days stored prior to planting					
		15	32 ^b	69	94	132	172
	Control - dry untreated seeds	83	75	Emergence ^c		87 ^d	83
54-1	5 per cent water	86	74	76	68	66 ^d	48 ^d
54-41	Inhib. A 0.13 g. + 5 per cent water	82	61 ^d	67	68	46	24
	5 per cent water +						
54-11	Ceresan M 0.032 g.	85	81	74	79	59 ^d	0
54-20	" 0.065 g.	88	70	81	71	59 ^d	0
54-29	" 0.13 g.	70	37	41	47	36 ^d	0 ^e
	Inhib. A to Ceresan M, 1:1 + 5 per cent water						
54-13	Ceresan M 0.032 g.	86	78	94	74	52	28
54-22	" 0.065 g.	84	66	77	70	53	8
54-31	" 0.13 g.	31	13	17	16	0	--

^aDosages per 100 g. of seed.

^bSeedling emergence for this storage period read at 5 days after planting.

^cEmergence given as an average of plants in two replicates except "d".

^dEmergence given for only one replicate due to erratic germination.

^eReduction of loose smut was 98.2 per cent in the field.

All treatments in Table 11 have 15 per cent water added either as a saturated solution of Inhibitor A or as water alone. Addition of water equal to 15 per cent of seed weight reduces seed viability rapidly compared to the dry untreated control. Presence of the inhibitor alone (Treatment 54-44) gave greater reduction in emergence than did the 15 per cent water (Treatment 54-1 a).

Reduction in emergence because of the addition of a saturated solution of Inhibitor A would seem to indicate that at this moisture level and inhibitor dosage, Inhibitor A can be injurious to seed. Increased injury to seed due to Inhibitor A when used alone compared to the injury produced by water alone suggests that the inhibitor acts on the mercury compound and not on the seed itself. Effects of the mercury injury inhibition are again more evident at the higher Ceresan M dosages (Treatment 54-34, Table 11).

Four treatment combinations utilizing three dosage levels of Ceresan M; without water, with 5 per cent water, with Inhibitor A added plus 5 per cent water, and with 5 per cent total moisture added as a saturated solution of Inhibitor A gave various results (Table 12). Addition of 5 per cent water (Treatment 54-1) and storage resulted in a reduction in seed viability compared to the dry untreated control but it was neither as great nor as rapid as that caused by storage and 15 per cent water (Table 11, Treatment 54-1 a). Addition

Table 11. Greenhouse seedling emergence from barley seed given varying periods of storage following slurry treatments of three dosage levels of Ceresan M with 15 per cent water or with 15 per cent of a saturated solution of Inhibitor A added, 1954

Treatment number	Treatment ^a		Days stored prior to planting			
			15	32 ^b	70	94
	Control - dry untreated seeds		82	Emergence ^c		
				75	87	86
54-1 a	15 per cent water		79	70	1	0
	<u>Ceresan M</u>	<u>Inhibitor^d</u>				
54-44	none	present	65	13	0	--
54-12	0.032 g.	none	80	61 ^e	1	0
54-16	0.032 g.	present	76	9 ^e	15	5 ^e
54-21	0.065 g.	none	80	50	0	--
54-25	0.065 g.	present	75	22 ^e	2 ^e	0
54-30	0.13 g.	none	34	1	1	1
54-34	0.13 g.	present	62	4 ^e	3 ^e	0

^aDosage per 100 g. of seed.

^bEmergence for this storage period read at five days after planting.

^cEmergence given as an average of plants in two replicates except "e".

^dA saturated solution of Inhibitor A was added equal to 15 per cent of seed weight giving approximately 0.1995 g. of actual inhibitor per 100 g. seed.

^eEmergence given for only one replicate due to erratic germination.

of moisture to Ceresan M at the highest dosage level increased injury in comparison to the same dosage level applied dry (Table 12, Treatments 54-29 and 54-28).

Ceresan M at 0.13 g. with 5 per cent water (Treatment 54-29), however, resulted in a 98.2 per cent reduction of loose smut. The same Ceresan M dosage dry (Treatment 54-28) reduced the smut only 48.7 per cent.

Inhibitor A in an inhibitor to Ceresan M ratio of 1:1 (Table 10) did not compare well with even Ceresan M plus 5 per cent water alone, (Table 12, Treatments 54-11, 54-20 and 54-29) except for the 173-day storage period which had an average of 28 and 8 seedlings (Treatments 54-13 and 54-22). Comparable Treatments 54-11 and 54-20 with no inhibitor had no viable seed after 173 days.

Greater injury inhibition, except in Treatment 54-15, resulted from the use of 5 ml. of a saturated solution of Inhibitor A than was obtained from the treatments using an Inhibitor A to Ceresan M ratio of 1:1 (Tables 10 and 12). Treatment 55-33 using Ceresan M at 0.13 g. and Inhibitor A in an inhibitor to Ceresan ratio of 0.5:1 resulted in nearly the same degree of injury inhibition as Treatment 54-32 (Table 9). Treatment 54-33 resulted in complete control of loose smut while Treatment 54-32 reduced smut only 39.8 per cent.

Addition of approximately 0.2256 g. of actual Inhibitor B as a dilution of the saturated solution, Table 13, resulted in

Table 12. Greenhouse seedling emergence from barley seed given varying periods of storage following slurry treatments with three dosage levels of Ceresan M using four treatment combinations, with and without Inhibitor A, 1954

Treatment number	Treatment ^a	Days stored prior to planting					
		15	32 ^b	70	94	133	173
	Control - dry untreated seeds	83	75	Emergence ^c		87 ^d	83
54-1	5 per cent water	86	74	76	68	66 ^d	48 ^d
54-10	Ceresan M 0.032 g.	88	79	81	79	56	0
54-19	" 0.065 g.	86	72	86	84	63	0
54-28	" 0.13 g.	78	71	83	81	72 ^d	5
	5 per cent water +						
54-11	Ceresan M 0.032 g.	85	81	74	79	59 ^d	0
54-20	" 0.065 g.	88	70	81	71	59 ^d	0
54-29	" 0.13 g.	70	37	41	47	36 ^d	0
	1:1 Inhib. A to Ceresan M + 5 per cent water						
54-13	Ceresan M 0.032 g.	86	76	82	74	52	28
54-22	" 0.065 g.	84	66	77	70	53	8
54-31	" 0.13 g.	31	13	17	16	0	0

^aDosage given in grams per 100 g. of seed.

^bEmergence for this storage period read five days after planting.

^cEmergence given as an average of plants in two replicates except "d".

^dEmergence given for only one replicate due to erratic germination.

Table 12 (Continued).

Treatment		<u>Days stored prior to planting</u>					
number	Treatment ^a	15	32 ^b	70	94	133	173
	Inhibitor A saturated solution 0.0665 g. ^e + 5 per cent T.M.A.						
54-15	Ceresan M 0.032 g.	85	71 ^d	80	72	67 ^d	19
54-24	" 0.065 g.	82	59	82	74	67 ^d	25
54-33	" 0.13 g.	75	59	62	55	68 ^d	36

^eThe calculated amount of actual inhibitor added in 5 ml. of a saturated solution.

mercury injury inhibition almost equal to that achieved with Inhibitor A in Treatments 54-14, 54-23 and 54-32 (Table 9) and greater inhibition than with dosages of Inhibitor A in Treatments 54-15, 54-24 and 54-33 (Table 12).

Reduction of loose smut by the Inhibitor B treatments in Table 13 was not above 68.2 per cent (See Appendix). Increasing the Ceresan M dosage while controlling mercury injury through the use of Inhibitor B, however, might make possible complete control of loose smut while not reducing seed viability below an acceptable level.

A comparison of greenhouse seedling emergence from seed given varying periods of storage following slurry treatment using Ceresan M 0.13 g. with three different levels of Inhibitor A and one of Inhibitor B (Figure 1) indicated seed

Table 13. Greenhouse seedling emergence from barley seed given varying periods of storage following slurry treatments with three dosage levels of Ceresan M in three treatment combinations, with and without a solution of Inhibitor B, 1954

Treatment number	Treatment ^a	Days stored prior to planting					
		15	32 ^b	70	94	133	173
	Control - dry untreated seeds	83	82	Emergence ^c			
54-1	5 per cent water	86	74	76	68	66 ^d	48 ^d
54-46	Inhib. B sat. sol. 0.2256 g. + 5 per cent T.M.A.	85	86	84	76	70 ^d	53
54-10	Ceresan M 0.032 g.	88	79	81	79	56	0
54-19	" 0.065 g.	86	72	86	84	63	0
54-28	" 0.13 g.	78	71	83	81	72 ^d	5
	5 per cent water +						
54-11	Ceresan M 0.032 g.	85	81	74	79	59	0
54-20	" 0.065 g.	88	70	81	71	59 ^d	0
54-29	" 0.13 g.	70	37	41	47	36 ^d	0
	5 per cent T.M.A. + Inhib. B (from sat. sol.) ^e						
54-18	Ceresan M 0.032 g.	88	85	78	81	71	69
54-27	" 0.065 g.	87	84	82	80	71	65
54-36	" 0.13 g.	63	79	68	75	66 ^d	58

^aDosages given in grams per 100 g. of seed.

^bEmergence for this storage period read five days after planting.

^cEmergence given as an average of plants in two replicates except "d".

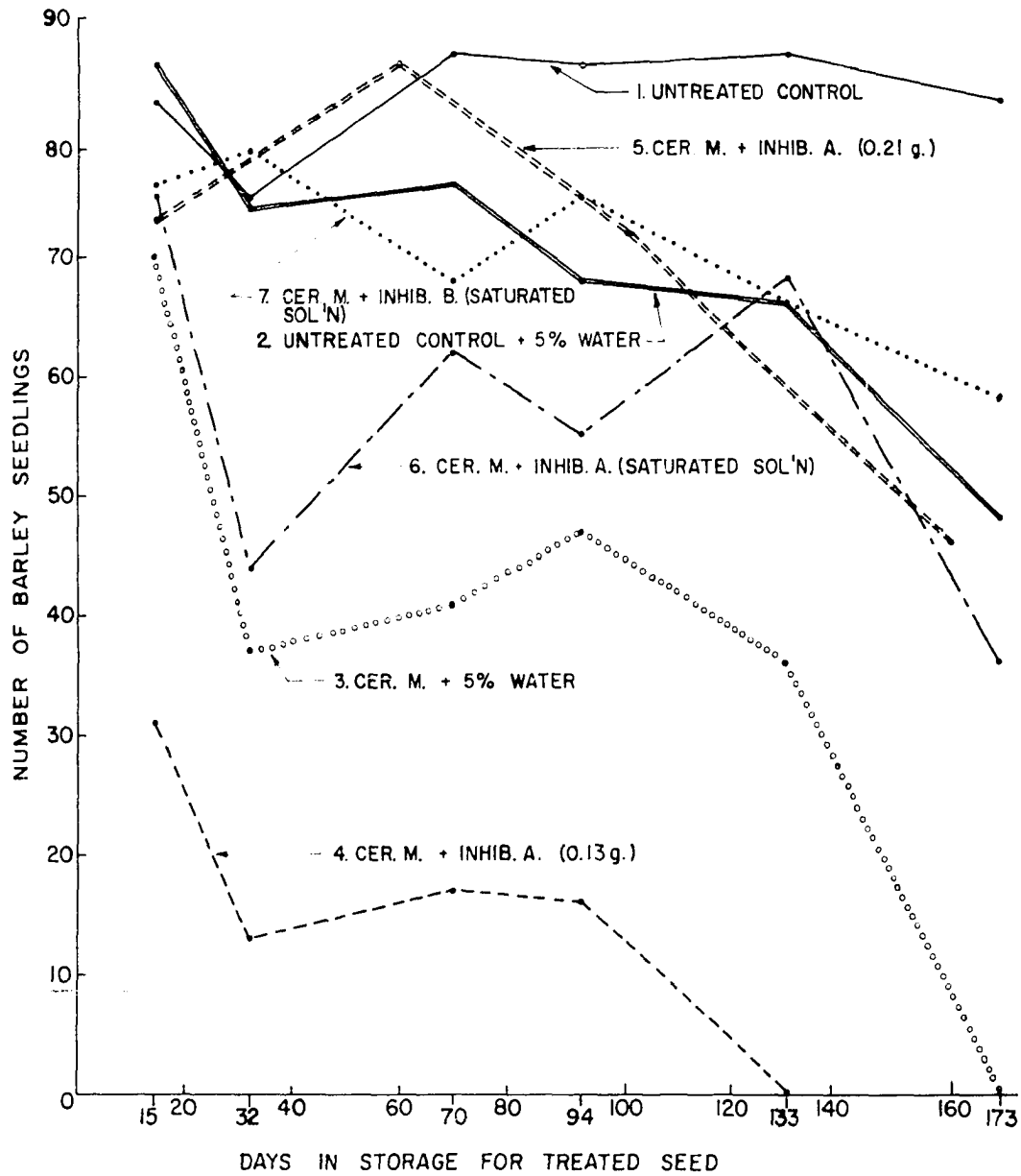
^dEmergence given for only one replicate due to erratic germination.

^eSee the Appendix for treatment details.

Figure 1. Greenhouse seedling emergence from barley seed given varying periods of storage following treatment with Ceresan M 0.13 g. in different moisture and inhibitor combinations, 1954

Curve number	Treatment number	Treatment
1		Control - dry untreated seeds
2	54-1	5 per cent water
3	54-29	Ceresan M, plus 5 per cent water
4	54-31	Ceresan M, plus Inhib. A in 1:1 ratio with Ceresan M, 5 per cent water
5	54-32	Ceresan M, plus Inhib. A in 1.6:1 ratio with Ceresan M, 5 per cent water
6	54-33	Ceresan M, plus sat. sol. Inhib. A, 5 per cent T.M.A.
7	54-36	Ceresan M, plus sol. Inhib. B, 5 per cent T.M.A. ^a

^aSee Appendix for treatment details.



viability was reduced to zero by 133 days with Ceresan M plus Inhibitor A (Curve 4, Treatment 54-31) and by 173 days using Ceresan M with 5 per cent water and no inhibitor (Curve 3, Treatment 54-29). Ceresan M with Inhibitor A (Curve 5, Treatment 54-32) after 162 days of sealed storage had an average emergence of 46 seedlings. The remaining treatments after 173 days of storage had an average emergence of 36 seedlings (Curve 6, Treatment 54-33) and 58 seedlings (Curve 7, Treatment 54-36). The dry untreated control (Curve 1) after 173 days gave an average emergence of 83 seedlings, while 5 per cent water alone (Curve 2, Treatment 54-1) had emergence reduced to an average of 48 seedlings compared to zero emergence from Treatment 54-29 (Curve 7) at that time. Although greenhouse seedling emergence tests were not made at the time of field planting, greenhouse emergence data indicated all treatments except 54-29 (Curve 4) and 54-31 (Curve 3) should have had an average emergence of greater than 50 seedlings. At the time of field planting all treatments had received 103 days of sealed storage except 54-32 which received 92 days. The greatest amount of injury inhibition resulted from use of Inhibitor B with Ceresan M (Curve 7, Treatment 54-38). Reduction of loose smut, however, was only 68.2 per cent. Treatment 54-33, Curve 6, which had an average emergence of 36 seedlings after 173 days of storage completely controlled loose smut while Treatment 54-29

Table 14. Greenhouse seedling emergence from barley seed given varying periods of storage following slurry treatments with three dosage levels of Ceresan 2 per cent, with and without Inhibitor A, 1954

Treatment number	Treatment ^a	Days stored prior to planting					
		15	32	70	94	133	173
				Emergence ^b			
	Control - dry untreated seeds	83	82	87	86	87 ^c	83
54-1	5 per cent water	86	74	87	68	66 ^c	48 ^c
54-1	Ceresan 2 0.081 g. per cent	86	89	90	84	82	72
54-4	" 0.162 g.	80	88	85	89	87	72
54-7	" 0.325 g.	86	85	86	87	79	74
	5 per cent water +						
54-2	Ceresan 2 0.081 g. per cent	83	83	86	85	78 ^c	73
54-5	" 0.162 g.	75	82	84	75	74 ^c	58
54-8	" 0.325 g.	83	80	72	66	54	41
	Inhib. A to Ceresan 2 per cent 0.8:1 + 5 per cent water						
54-3	Ceresan 2 0.081 g. per cent	88	83	81	81	44	55
54-6	" 0.162 g.	83	83	80	74	57	62
54-9	" 0.325 g.	81	67	69	75	61	71 ^c

^aDosages given in grams per 100 g. of seed.

^bEmergence given as an average of plants in two replicates, except "c".

^cEmergence given for only one replicate due to erratic germination.

(Curve 3) with no greenhouse emergence after 173 days, caused a 98.2 per cent reduction in smut when planted after 103 days of storage.

Ceresan 2 per cent was used at three dosage levels in three combinations: without water, with 5 per cent water and with Inhibitor A and 5 per cent water (Table 14). Reduction in greenhouse seedling emergence to somewhat below that obtained by the dry untreated control resulted from dry application of Ceresan 2 per cent. Addition of 5 per cent water caused some additional emergence reduction which was most noticeable at the highest dosage (Treatment 54-9). Reduction in loose smut among the treatments using 5 per cent water and no inhibitor ranged from 18.6 to 84 per cent; Treatment 54-8 produced the greatest reduction. Treatments using Inhibitor A ranged from 0 to 71.7 per cent reduction in loose smut; Treatment 54-9 showed the greatest reduction.

1955 Results

Treatments in 1955 were made on two different lots of barley: Variety 1 (Vantage barley) in which the dry untreated controls averaged 1.5 smutted heads per rod row in 12 replicates with a range of 0 to 7 smutted heads per row; Variety 2 (mixed barley varieties spray-innoculated with loose smut) in which the dry untreated controls averaged 5 smutted heads per rod row with a range of 1 to 9 smutted heads per row.

All treatments resulted in an unsatisfactory reduction of loose smut, less than 91 per cent. The greatest reduction in Variety 1 was 60 per cent, reduction ranging from none to 60 per cent. The same treatments made on Variety 2 resulted in no reduction of smut with the exception of one treatment which had a 12 per cent reduction. Results for 1955 did not follow trends established in prior years. Treatments are described in detail in the Appendix.

DISCUSSION

The slurry method of applying materials for the control of loose smut of barley was investigated as a means of avoiding the difficulties inherent in treatments which use large amounts of water. This method as commonly used on small grains has been successful only in the control of fungi on the surface or in the pericarp of the seeds. The loose smut organism, however, invades the more protected tissues of the embryo and has not been controlled previously by slurry treatments.

Several problems are involved in adaptation of the slurry to control of loose smut. Selection of materials which will control the fungus is important, so is the effect of dosage on seed viability. In sealed storage, the relationships between treatment material, the percentage of moisture added and the time required to permit the treatment material to reach the pathogen mycelium must be established. While the moisture-treatment material effects on seed viability can be measured easily by greenhouse seedling emergence tests, only through field testing can the sealed storage time required for control of the disease be determined.

No change in viability occurred in seed treated with Ceresan M at 0.032 g. when 5 per cent water was added (Table 12). Differences in seedling emergence between dry applications of chemicals and those with the

addition of a constant amount of water became apparent at the higher dosage levels and as time in sealed storage increased. For the organic mercury compounds seedling emergence decreased as dosage level and storage time increased.

Only one treatment with 15.6 per cent total moisture added resulted in satisfactory emergence and complete control of loose smut. Other treatments utilizing the addition of 15 per cent moisture either as water or as a saturated solution of Inhibitor A caused such a rapid drop in seed viability that the treatments were discontinued and not taken to the field for testing (Treatments 54-16, 54-21, 54-25, 54-30, 54-34, 54-44).

The best control in most cases was obtained from treatments using an addition of 5 per cent moisture (or total moisture added). However, one treatment using 2.5 per cent moisture (54-35) did reduce loose smut 91.2 per cent. This treatment with a longer period of sealed storage might have resulted in control without exceeding a permissible reduction in seed viability.

Acetone alone at 15 per cent of the seed weight resulted in a 93 per cent reduction in loose smut but caused a marked reduction in seed viability (Treatment 53-5). A 1:1 solution of acetone and water used as a solvent resulted in a greater reduction in seed viability at a given treatment level than

did water alone but gave complete control of loose smut when used with Ceresan M 0.13 g. and no inhibitor (Treatment 53-21). The same Ceresan dosage with 5 per cent water resulted in the following reductions of loose smut: Treatment 53-19, 81 per cent, Treatment 54-29, 98.2 per cent.

Organic mercury compounds in various treatment combinations applied as low moisture slurries achieved the greatest reduction in loose smut in these experiments. Reductions in loose smut of 91 per cent or better were obtained through the use of Ceresan M, P.D.R. "Regular" (Treatment 52-11), Phillips OSS-11148, Puratized C4-10, and Velsicol 50-C5-46. Phillips OSS-11148 (Treatments 53-44 and 53-45) and Velsicol 50-C5-46 (Treatment 53-61) performed well and are no doubt worth further investigation even though they were not tested after 1953. P.D.R. "Regular", a combination of Ceresan 2 per cent and Inhibitor A, was used in one slurry treatment which gave complete control of loose smut but did not perform as well in the following year, so was discarded.

With the exceptions of Treatment 52-11 with P.D.R. "Regular" and Treatment 53-61 with Velsicol 50-C5-46 (99 per cent reduction), the only treatment combinations which eliminated loose smut included Ceresan M at 0.13 g. or 0.26 g. Ceresan M at 0.13 g. was used with a number of treatment modifications, three of which resulted in complete control of loose smut (Treatments 53-21, 53-24 and 54-33).

This dosage of Ceresan M in different treatment combinations resulted in loose smut reductions from 39.6 to 100 per cent. The three treatments resulting in the least reduction in loose smut with this dosage of Ceresan M had inhibitors added (Treatments 53-26, 54-32 and 54-36).

Two treatments with Ceresan M at 0.26 g. eliminated loose smut (Treatments 53-7 and 53-28); a third treatment reduced smut by 91 per cent. The variation in control obtained with a given chemical dosage on samples from the same lot of seed may be due to an interaction of the treatment chemical and inhibitor at certain levels or may be influenced by other variables. Differences in the response to a given treatment on different seed varieties grown in the same place, and on the same variety of seed grown in different locations or in different years has been established by Arny and Leben (1).

Dry applications of Ceresan M 0.13 g. (Treatment 54-28) did not provide satisfactory reduction in loose smut while the addition of 5 per cent water to the Treatment (54-29) resulted in smut reduction of 98.2 per cent. Both treatments were given 103 days of sealed storage at room temperature. Sublimation alone of the mercury compounds in Ceresan M apparently was not sufficient to effect control. Added water diffusing into the seed must be involved in some manner in the movement of the fungicide. Ceresan M is not, however,

considered to be water soluble, though it is soluble in acetone (8). The solubility of some organic mercury compounds in acetone and the relative lack of injury to seed by acetone were the reasons for using it alone, and with water.

An important consideration in the use of mercury compounds to control loose smut is their effectiveness against a wide range of seed and soil borne organisms. Though not experimentally demonstrated in this investigation, sufficient mercury from the treatment should remain on the seed surface to provide protection against seed rotting fungi and possible protection through sublimation against root rotting fungi.

The experimental mercury inhibitor compounds A and B were used in treatment combinations with Ceresan M and Ceresan 2 per cent. Inhibitor A was originally used with Ceresan 2 per cent as a seed potato treatment by Dr. C. S. Reddy¹. Only one Treatment (52-11) using Inhibitor A and Ceresan 2 per cent resulted in successful control of loose smut. All others did not provide satisfactory control. The combinations of Inhibitor A and Ceresan 2 per cent used did result in some increase in seedling emergence at the higher dosage levels when compared with the emergence resulting from treatments using the same dosages of Ceresan 2 per cent

¹Reddy, C. S., Professor, Department of Botany and Plant Pathology, Iowa State University, Ames, Iowa. A dust treatment for seed potatoes. Private communication. 1951.

and moisture but without the inhibitor (Table 14). Either inhibitor without Ceresan M or Ceresan 2 per cent at any level of added moisture caused a greater reduction in seedling emergence than did the same amount of water added alone, indicating that inhibitors added with only water have a deleterious effect on seed viability.

Used in certain proportions with Ceresan M and Ceresan 2 per cent, Inhibitors A and B reduced mercury injury. This is most obvious at the higher dosage levels of the fungicide. The effects of injury inhibition also become more noticeable as time in sealed storage increased.

A proper balance between the amount of inhibitor and organic mercury is important; some combinations reduced injury while others did not (Table 12). This relationship should be investigated further. Some combinations of inhibitors and Ceresan M, especially at the higher Ceresan M dosages, and as time in sealed storage increased, resulted in higher seedling emergence than was obtained by either the inhibitor or mercury compound alone. The injury inhibitory effect could be due to the formation of chemical complexes in which the mercury is in a form less injurious to the host protoplasm yet still active against fungus mycelium.

Reduction in loose smut by a given treatment is the product of a number of variables. A complex of such variables produced the differences in the numbers of smutted

heads obtained from samples of the same seed lot planted on different dates. In 1952 dry untreated controls planted on April 19 resulted in an average of 22.5 smutted heads per rod row with a range of 3 to 35 smutted heads per row. The dry control from the same seed lot planted May 24 produced an average of only 5.5 smutted heads per rod row with a range of 3 to 11 smutted heads per row. Under certain conditions of temperature and moisture it is apparent that the host plant can elongate fast enough to escape establishment of the pathogen mycelium in the apical meristem where it otherwise would be carried along during elongation and ultimately be in position to replace the inflorescence.

Essential for effective evaluation of seed treatments for control of loose smut is seed which has a sufficiently high level of smut infection. The supply of such seed should be large enough to make possible the number of variations in treatments over the time required to develop an effective control. A low smut incidence in seed used for testing treatments was no doubt responsible for some of the variation found.

With further work there is no doubt that a practical slurry treatment which will control loose smut of barley can be developed, a treatment which will be an improvement over the current treatment methods.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to develop a control for loose smut of barley without the disadvantages found in the current methods which are: wet swollen seed requiring special handling and the necessity for an additional fungicide treatment to control surface borne seed pathogens. Two methods of applying chemicals were used; the aqueous soak and the slurry. The slurry method using small additions of water or other solvents with the chemicals avoided the above disadvantages and was the method most used. Field data were taken in 1952, 1953, 1954, and 1955.

Thirteen soak treatments were field tested, three of which provided a 91 per cent or greater reduction in loose smut. The treatments were:

1. Water alone with a 46-hour soak, 91 per cent reduction (Treatment 53-1 a);
2. P.D.R. "C" with a 24-hour soak, 97 per cent (Treatment 53-1 b);
3. Spergon 46 per cent wettable with a 40-hour soak preceded by a 6-hour presoak in water alone, 100 per cent reduction (Treatment 53-2).

Of one hundred and eleven slurry treatments field tested, the following six resulted in elimination of loose smut, a seventh in a 99 per cent reduction:

1. P.D.R. "Regular" 0.06 g.^a plus 5 per cent 1:1 acetone and water (Treatment 52-11);

^aAll dosages per 100 g. of seed.

2. Ceresan M 0.13 g. plus 5 per cent 1:1 acetone and water (Treatment 53-21);
3. Ceresan M 0.13 g. plus Inhibitor A saturated solution, 5 per cent T.M.A.^b (Treatment 54-33);
4. Ceresan M 0.13 g. plus Inhibitor A saturated solution, 15.6 per cent T.M.A. (Treatment 53-24);
5. Ceresan M 0.26 g. plus 5 per cent water (Treatment 53-27);
6. Ceresan M 0.26 g. plus Inhibitor A, 5 per cent water (Treatment 53-28);
7. Velsicol 50-C5-46 0.26 g. plus 5 per cent water (Treatment 53-61).

The following treatments provided a 96 per cent reduction in loose smut:

1. Ceresan M 0.13 g. plus Inhibitor B saturated solution, 5 per cent T.M.A. (Treatment 53-25);
2. Phillips OSS-11148 0.13 g. plus 5 per cent water (Treatment 53-44);
3. Phillips OSS-11148 0.521 g. plus 5 per cent water (Treatment 53-45).

Mercury compounds were found to give the best control of loose smut with the added advantage of being effective against a wide range of seed borne pathogens. Seed injury resulting from the mercury was reduced by the two experimental mercury injury inhibitors added to the chemicals in the proper proportions. Effectiveness of the injury inhibition became more evident as the mercury dosages and storage times increased.

^bT.M.A., total moisture added indicates all or part of the moisture added was from a saturated solution of one of the inhibitors.

Sealed storage to allow the chemical and solvent time to diffuse into the seed is an important part of this method and the time required to obtain control without reducing seed viability beyond permissible limits must be determined. A practical slurry treatment which will control loose smut of barley can be developed with further work.

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APPENDIX

Table 15. 1952 soak treatments

Treatment number	Chemical ^a	Soak period in hours	Dosage ^b	Emulsifier B	Percentage reduction of loose smut
52-1	C-6146	24	9:10,000	9:10,000	66
52-2	Inhibitor A 50 per cent	"	1:1,000	-----	77
52-3	P.D.R. "C"	"	1:1,000	-----	87.6
52-4	P.D.R. "Regular"	"	1:1,000	-----	84
52-5	P.D.R. "Regular"	"	1:800	-----	85
52-6	P.D.R. "Regular"	10	1:400	-----	73
52-7	Phygon XL	24	1:375	-----	40
52-8	Spergon 96 per cent	"	4:1,000	0.08:1,000	88.7

^aAll chemicals in the Appendix are alphabetized with the exception of water.

^bParts of chemical to parts of water.

Table 16. 1952 slurry treatments

Treatment number	Chemical ^a	<u>Dosage per 100 g. seed</u>		Days sealed storage ^b	Percentage reduction of loose smut
		Chemical	Solvent		
52-9	Ceresan M ½ oz./bu.	0.0651 g.	-----	4	8
52-10	E.C. 1182 3.8 per cent active	10 ml. 6:10,000 E.C. 1182 and acetone sol.	acetone	14	0
52-11	P.D.R. "Regular"	0.06 g.	5 ml. 1:1 acetone and water sol.	"	100
52-12	P.M.A.S.	0.13 ml.	3 ml. water	42	0

^aAll chemicals in the Appendix are alphabetized with the exception of water.

^bDays in sealed storage prior to planting in the field.

Table 17. 1953 soak treatments

Treatment number	Chemical	Soak period in hours	Dosage	Emulsifier B	Percentage reduction of loose smut
53-1	Water	24	-----	-----	72
53-1 a	Water	46	-----	-----	91
53-1 b	P.D.R. "C"	24	1:1,000	-----	97
53-2	Spergon 46 per cent wettable	6 hr. presoak 40 hr. soak	2:1,000	-----	100
53-3	Spergon 96 per cent	24	4:1,000	0.08:1,000	87

Table 18. 1953 slurry treatments

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-4	Water	5 ml.	-----	-----	81	32
53-4 a	Acetone	8 ml.	-----	-----	96	28
53-5	"	15 ml.	-----	-----	81	93
53-6	BB 71 (liquid)	3.75 ml. 10:128 ^a	-----	1.25 ml. water	97	23
53-7	"	2.5 ml. 10:118	-----	2.5 ml. water	82	46
53-8	"	2.5 ml. 10:128	-----	"	97	28
53-9	"	3.75 ml. 10:118	-----	1.35 ml. water	82	22
53-10	"	5 ml. 10:118	-----	-----	82	50
53-11	"	5 ml. 10:128	-----	-----	97	56
53-12	Ceresan M $\frac{1}{2}$ oz./bu.	0.0651 g.	-----	5 ml. water	93	42

^aChemical and water.

Table 18 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-13	Ceresan M $\frac{1}{2}$ oz./bu.	0.0651 g.	-----	5 ml. 1:1 acetone and water	95	91
53-14 ^b	"	"	-----	5 ml. water	93	53
53-15	"	"	0.0521 g.	"	"	46
53-16 ^c	"	"	3.91 ml. sat. sol. Inhib. A	11.74 ml. water	92	68
53-17	"	"	1 ml. 100:121 sat. sol. Inhib. B and water	4 ml. water	"	41
53-18	"	"	1 ml. 100:121 sat. sol. Inhib. B and water	1.5 ml. water 2.5 ml. acetone	95	93

^bJar rinsed with water, chemical plus 5 ml. water then added.

^cSlurried 2½ hrs., dried 30 min., rebottled, sealed.

Table 18 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-19	Ceresan M 1 oz./bu.	0.1302 g.	-----	5 ml. water	93	81
53-20 ^d	"	"	-----	"	"	91
53-21	"	"	-----	5 ml. 1:1 acetone and water	95	100
53-22	"	"	Inhib. A 0.1042 g.	5 ml. water	93	93
53-23 ^e	"	"	"	5 ml. 1:1 acetone and water	95	91
53-24	"	"	7.825 ml. sat. sol. Inhib. A	7.825 ml. water	92	100
53-25	"	"	2 ml. 100:1.21 sat. sol. Inhib. B and water	0.5 ml. water 2.5 ml. acetone	95	96

^dThe jar is rinsed with water, the chemical added with 5 ml. water and allowed to sit 24 hrs. prior addition of seed.

^eThe jar is rinsed with 1:1 acetone-water solution.

Table 18 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-26	Ceresan M 1 oz./bu.	0.1302 g.	2 ml. 100:1.21 sat. sol. Inhib. B and water	3 ml. water	92	68
53-27	Ceresan M 2 oz./bu.	0.2604 g.	-----	5 ml. water	93	100
53-28	"	"	Inhib. A 0.2085 g.	"	"	100
53-29	"	"	2 ml. 200:1.89 sat. sol. Inhib. B and water	3 ml. water	92	91
53-30	8-Hydroxy- quinolate sulfate $\frac{1}{2}$ oz./bu.	0.065 g.	-----	5 ml. water	78	16
53-31	8-Hydroxy- quinolate sulfate 1 oz./bu.	0.1302 g.	-----	5 ml. water	"	0

Table 18 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-32	8-Hydroxy- quinolate sulfate 1½ oz./bu.	0.1952 g.	-----	5 ml. water	78	40
53-33	N-244 (liquid)	4 ml. 1:1,000 N-244 and acetone	-----	4 ml. acetone	98	48
53-34	"	8 ml. 1:1,000 N-244 and acetone	-----	-----	"	16
53-35	N-521 (liquid)	4 ml. 1:1,000 N-521 and acetone	-----	4 ml. acetone	"	3
53-36	"	8 ml. 1:1,000 N-521 and acetone	-----	-----	"	63

Table 18 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-37	Panogen Liquid Seed Disinfectant 3/4 oz./bu.	5 ml. 50:2,527 Panogen and water	-----	-----	96	31
53-38	Panogen Liquid Seed Disinfectant 1 1/2 oz./bu.	5 ml. 100:2,527 Panogen and water	-----	-----	"	58
53-39	Panogen Liquid Seed Disinfectant 3 oz./bu.	5 ml. 100:1,264 Panogen and water	-----	-----	"	77
53-40	Panogen S Liquid 1/2 oz./bu.	1.66 ml. 5:379 Panogen S and water	-----	2.5 ml. water	78	9
53-41	Panogen S Liquid 1 oz./bu.	3.34 ml. 10:374 Panogen S and water	-----	1.8 ml. water	"	1

Table 18 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-42	Panogen S Liquid 1½ oz./bu.	5 ml. 15:369 Panogen S and water	-----	-----	78	16
53-43 ^f	P.D.R. "Regular"	0.06 g.	-----	2.5 ml. acetone 2.5 ml. water	93	73
53-44	Phillips OSS-11148	0.1302 g.	-----	5 ml. water	90	96
53-45	"	0.521 g.	-----	"	"	96
53-46	"	0.5208 g.	-----	"	"	77
53-47	P.M.A.S. ½ oz./bu.	1.66 ml. 5:379 P.M.A.S. and water	-----	3.54 ml. water	95	42
53-48	P.M.A.S. ¾ oz./bu.	2.5 ml. 75:3,765 P.M.A.S. and water	-----	2.5 ml. water	"	10

^fThe chemical is dissolved in acetone and brought up to volume with water.

Table 18 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-49	P.M.A.S. 1 oz./bu.	3.33 ml. 10:374 P.M.A.S. and water	-----	1.68 ml. water	95	41
53-50	P.M.A.S. 1 oz./bu.	6 ml. 13:600 P.M.A.S. and water	-----	-----	79	46
53-51	P.M.A.S. 1½ oz./bu.	5 ml. 15:369 P.M.A.S. and water	-----	-----	95	18
53-52	Puratized C4-10 1½ oz./bu.	0.1953 g.	-----	5 ml. 1:1 acetone and water	90	91
53-53	Puratized C13-1212 1/8 oz./bu.	0.0163 g.	-----	5 ml. water	92	41
53-54	Puratized C13-1212 ¼ oz./bu.	0.0325 g.	-----	"	"	37

Table 18 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
53-55	Puratized C13-1212 $\frac{1}{2}$ oz./bu.	0.0651 g.	-----	5 ml. water	92	54
53-56	Puratized C15-127 $\frac{1}{2}$ oz./bu.	"	-----	"	"	69
53-57	Puratized C15-127 1 oz./bu.	0.1302 g.	-----	5 ml. water	"	54
53-58	Puratized C15-127 2 oz./bu.	0.2604 g.	-----	"	"	65
53-59	Velsicol 50-C5-46 $\frac{1}{2}$ oz./bu.	0.0651 g.	-----	"	"	60
53-60	Velsicol 50-C5-46 1 oz./bu.	0.1302 g.	-----	"	"	58
53-61	Velsicol 50-C5-46 2 oz./bu.	0.2604 g.	-----	"	"	99

Table 19. 1954 slurry treatments

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
54-1	Water	5 ml.	-----	-----	103	27.4
54-1 a	"	15 ml.	-----	-----	"	----
54-1 b	Ceresan 2 per cent 5/8 oz./bu.	0.081 g.	-----	-----	102	1
54-2	"	"	-----	5 ml. water	"	18.6
54-3	"	"	0.065 g. Inhib. A	"	"	0
54-4	Ceresan 2 per cent 1 1/4 oz./bu.	0.162 g.	-----	-----	"	16.8
54-5	"	"	-----	5 ml. water	"	38
54-6	"	"	0.13 g. Inhib. A	"	"	46.9
54-7	Ceresan 2 per cent 2 1/2 oz./bu.	0.325 g.	-----	-----	"	20.4

Table 19 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
54-8	Ceresan 2 per cent 2½ oz./bu.	0.325 g.	-----	5 ml. water	102	84
54-9	"	"	0.26 g. Inhib. A	"	"	71.7
54-10	Ceresan M ¼ oz./bu.	0.032 g.	-----	-----	103	0
54-11	"	"	-----	5 ml. water	"	24
54-12*	"	"	-----	15 ml. water	"	--
54-13	"	"	0.0325 g. Inhib. A	5 ml. water	"	25.7
54-14	"	"	0.0512 g. Inhib. A	"	"	4.4
54-15	"	"	5 ml. sat. sol. Inhib. A	-----	"	54

All treatments marked by this symbol () were slurried 30 minutes, allowed to stand approximately 3½ hrs., spread out and air dried 30 minutes then rebottled and sealed.

Table 19 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
54-16*	Ceresan M $\frac{1}{4}$ oz./bu.	0.032 g.	15 ml. sat. sol. Inhib. A	-----	103	--
54-17	"	"	1 ml. sat. sol. Inhib. B	1.5 ml. water	"	25.7
54-18	"	"	2 ml. sat. sol. Inhib. B	3 ml. water	102	6.2
54-19	Ceresan M $\frac{1}{2}$ oz./bu.	0.065 g.	-----	-----	103	23.9
54-20	"	"	-----	5 ml. water	"	52.3
54-21*	"	"	-----	15 ml. water	"	--
54-22	"	"	0.065 g. Inhib. A	5 ml. water	"	46.9
54-23	"	"	0.104 g. Inhib. A	"	"	24

Table 19 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
54-24	Ceresan M ½ oz./bu.	0.065 g.	5 ml. sat. sol. Inhib. A	-----	103	57.5
54-25*	"	"	15 ml. sat. sol. Inhib. A	-----	"	--
54-26	"	"	1 ml. sat. sol. Inhib. B	1.5 ml. water	"	23.9
54-27	"	"	2 ml. sat. sol. Inhib. B	3 ml. water	102	41.6
54-28	Ceresan M 1 oz./bu.	0.13 g.	-----	-----	103	48.7
54-29	"	"	-----	5 ml. water	"	98.2
54-30*	"	"	-----	15 ml. water	"	--
54-31	"	"	0.13 g. Inhib. A	5 ml. water	"	--

Table 19 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
54-32	Ceresan M 1 oz./bu.	0.13 g.	0.21 g. Inhib. A	5 ml. water	103	39.8
54-33	"	"	5 ml. sat. sol. Inhib. A	-----	"	100
54-34*	"	"	15 ml. sat. sol. Inhib. A	-----	"	--
54-35	"	"	1 ml. sat. sol. Inhib. B	1.5 ml. water	"	91.2
54-36	"	"	2 ml. sat. sol. Inhib. B	3 ml. water	102	68.2
54-37	du Pont Liquid 244	2.5 ml. 1:49 Liquid 244 and water	-----	2.5 ml. water	103	54

Table 19 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
54-38	du Pont Liquid 244	2.5 ml. 1:49 Liquid 244 and sat. sol. Inhib. A	-----	2.5 ml. water	103	27.5
54-39	"	3.75 ml. 1:49 Liquid 244 and water	-----	1.25 ml. water	"	26
54-40	"	3.75 ml. 1:49 Liquid 244 and sat. sol. Inhib. A	-----	"	"	45.2
54-41	Inhibitor A (dry)	0.13 g.	-----	5 ml. water	"	70
54-42	"	0.21 g.	-----	"	92	47
54-43	Inhibitor A (saturated solution)	-----	5 ml. sat. sol.	-----	103	32.8

Table 19 (Continued).

Treatment number	Chemical	Dosage per 100 g. seed			Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent		
54-44*	Inhibitor A (saturated solution)	-----	15 ml. sat. sol.	-----	---	---
54-45	Inhibitor B (saturated solution)	1 ml. sat. sol.	-----	1.5 ml. water	103	18.6
54-46	"	2 ml. sat. sol.	-----	3 ml. water	102	16.8

Table 20. 1955 slurry treatments

Treatment number	Chemical	Dosage per 100 g. seed			Special modifications	Days sealed storage	Percentage reduction of loose smut
		Chemical	Inhibitor	Solvent			
55-1	Water	5 ml.	-----	-----	Variety 1 Variety 2	91 "	13 0
55-1 a	Ceresan M 1 oz./bu.	0.13 g.	-----	5 ml. water	Variety 1 Variety 2	" "	50 0
55-2	"	"	5 ml. sat. sol. Inhib. A	-----	Variety 1 Variety 2	" "	47 0
55-3	"	"	1 ml. sat. sol. Inhib. B	1.5 ml. water	Variety 1 Variety 2	" "	0 0
55-4	Ceresan 2 per cent 3 oz./bu.	0.39 g.	6 ml. sat. sol. Inhib. A	-----	Variety 1 Variety 2	" "	60 12
55-5	Inhibitor A (saturated solution)	-----	5 ml. sat. sol. Inhib. A	-----	Variety 1 Variety 2	" "	7 0
55-6	Inhibitor B (saturated solution)	-----	1 ml. sat. sol. Inhib. B	1.5 ml. water	Variety 1 Variety 2	" "	40 0